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Western Blot Analysis: Detection of Proteins using Chemiluminescence

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National Institutes of Health

Reagents

ECL Western Blotting Analysis System
Amersham Pharmacia, Cat. RPN2108
Glycine
BioRad, Cat. 161-0716
Hyperfilm ECL
Amersham Pharmacia, Cat. RPN2103H
Methanol
Non-fat Dry Milk (NFDM)
Carnation Brand, Grocery Store of Choice
PVDF Membrane
BioRad, Cat. 162-0181
NaCl
Mallinckrodt, Cat. 7581
Tris Base
BioRad, Cat. 161-0717
Tris, pH 7.5, 1 M
Quality Biological, Inc., Cat.351-006-100
Tween 20
Sigma, Cat. P1379

Preparation

10X Running Buffer

Tris Base	30.3 g	f.c. [250 mM, pH 8.3]
Glycine	144.2 g	f.c. [1.92 M]
Milli-Q water	1.0 L	

Store at 4˚C

1X Running Buffer

10X Running Buffer	100 ml	
Milli-Q Water	890 ml	
10% SDS	10 ml	f.c. [0.1%]

Transfer Buffer

Tris Base	3.03 g	f.c. [25 mM, pH 8.3]
Glycine	14.4 g	f.c. [192 mM]
Milli-Q water	1.1 L	

10X TTBS

1M Tris (pH 7.5)	500 ml	f.c. [0.5 M]
NaCl	87.66 g	f.c. [1.5 M]
Tween-20	10 ml	f.c. [1%]

*Bring volume to 1L with sterile distilled water
Store at 4˚C

1X TTBS

10X TTBS	100 ml
Milli-Q Water	900 ml

Store at 4˚C

Procedure

1. Run Western gels in pre-chilled 1X Running Buffer at 200V for 60 min in ice bucket.

2. Transfer to PVDF membrane
   a) Pre-wet PVDF in 50% MeOH starting at one end and slowly proceeding across membrane.
   b) Equilibrate in 1X Transfer Buffer ~ 5 min.
   c) Set-up transfer apparatus.
   d) Transfer in pre-chilled 1X Transfer Buffer at 100 V for 60 min with ice pack and apparatus in ice bucket during transfer.

3. Block membrane in 20 ml 5% NFDM, 1X TTBS for 60 min on shaker.

4. Detect membrane in 2 ml (1˚ Ab diluted in 5% NFDM, 1X TTBS) in sealed bag on rocker overnight.

5. Rinse membrane 3 x 5 min with 1X TTBS.

6. Detect membrane in 10 ml (2˚ HRP-conjugated Ab + 1xTTBS) for 60 min, shaking

7. Rinse membrane 1 x 15 min followed by 4 x 5 min with 1X TTBS.

8. Mix equal volume Detection solutions 1 & 2 (125 µl/cm^2 membrane).

9. Add detection mixture to membrane allowing surface tension to keep solution on membrane surface without spilling off sides.

10. Incubate for exactly 1 min at RT.

11. Drain off detection solution and wrap membrane in Saran Wrap, carefully removing air pockets.

12. Expose to Hyperfilm for desired length of time.